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STUDIES OF METAL COMPLEXES OF DRUG FUROSEMIDE AND AMINO ACIDS WITH COPPER (II).

KEYWORDS: Stability constant, ΔlogK and mixed ligand complexes.

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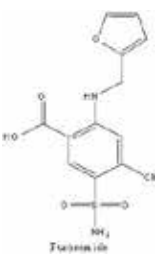
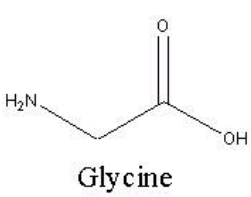
ABSTRACT *The stability constant of the mixed ligands complexes of copper (II) ion with drug furosemide as primary ligands and some amino acids were determined in 80 % (v/v) ethanol-water medium at 27°C fixed ionic strength 0.1M NaClO4 by computational programmed SCOGS.*

Introduction:

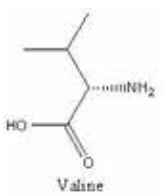


Furosemide, a sulfonamide type drug is an example of high-ceiling diuretic[1] & may be regarded as a derivative of anthranilic acid or o-aminobenzoic acid. It is used to cure hypertension and edema associated with congestive heart failure, cirrhosis and renal diseases.[2] Research on 5-sulfamoylanthranilic acid at the Hoechst laboratories in Germany showed them to be effective diuretics. The most active of a series of variously substituted derivative was furosemide. The chlorine and sulfonamide substitutions are features seen also in another diuretic such as thiazide. Because the molecule posses free carboxyl group, furosemide is a stronger acid than the thiazide diuretics. This drug is excreted primarily unchanged. A small amount of metabolism, however, can take place on the furan ring, which is substituted on the aromatic amino group. Furosemide has a saluretic effect[2, 3] ten times that of the thiazide diuretics



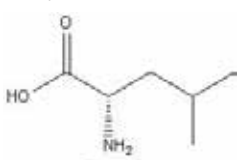
Glycine

All amino acids, commonly known as Magic 20 are polymer and regarded as building block of protein. Some amino acids are studied in this research [4]. Glycine (α-amino acetate), is the simplest, neutral, aliphatic, optically inactive non-essential, glycogenic aminoacid, [5-8]. It is isolated from protein and has characteristics sweet taste. Zwiter ionic structure some time called as betaine structure. It can be synthesized from CO_2 and NH_3 by glycine synthase or transamination of glyoxylate and in metabolism of serine and choline. It plays an important role in haeme synthesis.



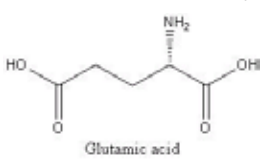
Valine

Valine (α- aminoiosovalerate), is essential amino acid[9-22]. It is widely distributed but rarely occurs in amount exceeding 10%. It is branched chain amino acid and can be derived from alanine by the introduction of two methyl group present on α - carbon atom. Ths is glycogenic. On deamination, it forms methyl-malonyl-CoA which can be converted to succinyl – CoA in place of two H atoms of the Methyl group.



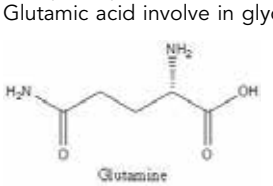
Leucine

Leucine[23] (α-amino isocaproate), is branched chain neutral essential ketogenic amino acid and forms an acetoacetate and acetate. It is taken up by brain and muscle.



Glutamic acid

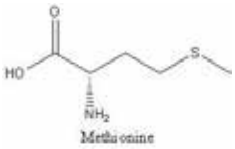
Glutamic acid[24] (α-aminoglutarate), is acidic non-essential glycogenic amino acid with one amino group and two carboxylic groups. It is parent compound of glutamine and widely occurs in protein.It takes part in transamination, transamidation and inter conversion of amino acids and also participate in ammonia transport and urea formation. Glutamic acid involve in glycogenic function.



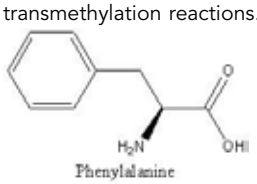
Glutamine

Glutamine (γ-amide of α-aminoglutarate**),** is acidic non-essential glycogenic amino acid[25, 26]. It is homologue of aspargine and constituent of folic acid. Basically it is used in higher animal for conjugation, detoxification of phenyl acetic acid and plant tissue.

Volume : 5 | Issue : 3 | March 2015 | ISSN - 2249-555X



Methionine[27] (α-amino-β-methylmercaptobutyrate), is essential glycogenic amino acid. It is the only common amino acid possessing an ether linkage. Cereals have sufficient quantity of methionine. Methionine is particularly important as a donor of methyl group in reaction known as transmethylation reactions.



Phenylalanine[28, 29] (α-amino-β-phenylpropionate), is aromatic essential glucogenic and ketogenic amino acid. Several abnormalities observed in phenylanine metabolism such as phenylketonaria and alkaptonaria. phenylketonaria and alkaptonaria are the inheritance abnormalities , in phenylketonaria, there is a black in hydroxylation of phenyl alanine to form tyrosine, this leads to mental retardation. Alkeptanaria, in this homogenstic acid is not further oxidised and excreted in urine. This leads to black urine.

Copper is a transition metal ion and is used by various enzymes in the body in different biochemical reactions. These reactions may be creating energy, decreasing the body's inflammatory blood clotting[32] etc. Copper is absorbed by the body at two main sites such as small intestine and stomach. Copper does not float through the blood stream as copper ion but is carried by proteins. Two main carrier proteins especially for copper are ceruloplasmin[33] and albumin; these can carry many things including copper. Copper is stored in proteins called metallothione.[34, 35] Enzymes are proteins specialized to assist in a chemical function. Copper is needed by enzymes as a helper in a chemical reaction. This function makes copper essential for cytochrome C oxidase, essential for energy and superoxide dismutase essential oxidative tissue damage etc.

In recent years it has been proved that transition metals like copper is essential for normal development and function of human cells. Disruption of copper metabolism causes severe neurodegenerative disease, such as Wilson's disease[36-40], and Menken's disease[41-43] with symptoms that range from psychiatric abnormalities and motor dysfunction, to poor temperature control and liver & kidney abnormalities.

Rossetti and Rossotti[44] defined complex, as a species formed by association of two or more simpler species, each capable of independent existence.

The formation of complex is not restricted to association between two ions of positive charges. A metal cation, a proton or another positively charged species may form complex with an electron donor, whether it is negatively charged[45-46]; electrically neutral or even positively charged. The ligand is referred as electron donor atoms or groups. This term is sometimes applicable to the molecule as a whole, which contains donor atoms or groups by means of which the molecule is attached to the central metal atom. When a ligand contains two or more donor atoms close to each other, the metal complex formed is said to be a "chelate"[47]

The most obvious feature of metal–chelate structure is the formation of heterocyclic ring, usually of five or six membered. Owing to the range of normal covalent bond angle, five and six membered rings are more stable. For a ring of single bond only the five-membered ring is usually most stable[48] where as a six membered rings have maximum stability, when there are two double bonds in the ring. The chelates have been extensively studied in the solid state as well as in the solution by many workers[49] due to their remarkable properties and high stability.

The present investigation deals with the study of stability constants of various metal chelates in 80% (v/v) ethanol-water medium. It is therefore; appropriate to mention the salient features of solution study.

Material and methods

The nitrates of transition metal ions copper, of Analar quality were obtained from B.D.H. (India). Metal ion was used in the form of their perchlorates to avoid the possibility of complex formation with anions. The perchlorates were prepared from the corresponding nitrates[51]. The concentration of metal ions was estimated by the standard procedures[52-54].

Sodium porchlorate (E.Merck) was dissolved in carbon dioxide free distilled water.

The solution of sodium hydroxide was also prepared in carbonate free distilled water by allowing the solution to stand for a long time till any carbonate if present precipitated. The solution was filtered and kept in a pyrex vessel, free from carbon dioxide and was used as titrant for the pH titration. As a routine, the solution was standardized at least once every day by titrating with standard oxalic acid solution.

Perchloric acid of Reidal (Germany) was used for the preparation of the stock solution. Its exact normality was obtained by titrating it conductometrically using standard sodium hydroxide solution.

Amino acids such as Glycine, arginine, tryptophan, Leucine, Glutamic acid, Glutamine, Methionine and Phenyl alanine obtained either from Merck (Germany) or Fluka (Germany) were prepared by dissolving Analar grade sample in 80% (v/v) ethnol – water medium.

Drugs such as Furosemide were prepared by dissolving as received as sample in 80% (v/v) ethanol-water medium. Drugs samples in pure form were obtained from pharmacy industries.

The experimental procedure, in the study of ternary chelates by the potentiometric titration technique, involves the titrations of carbonate free solution of

I	Free $HClO_4$ (A)
II	Free $HClO_4$ (A) + Ligand (D)
III	Free $HClO_4$ (A) + Ligand (D) + Metal ion (M)
IV	Free $HClO_4$ (A) + Ligand (R)
V	Free $HClO_4$ (A) + Ligand (R) + Metal ion (M)
VI	Free $HClO_4$ (A) + Ligand (D) + Ligand (R)+ Metal ion (M)

against standard sodium hydroxide, where D and R, are the two ligands. The ionic strength of the solutions was maintained constant i.e. 0.1 M by adding appropriate

Volume : 5 | Issue : 3 | March 2015 | ISSN - 2249-555X

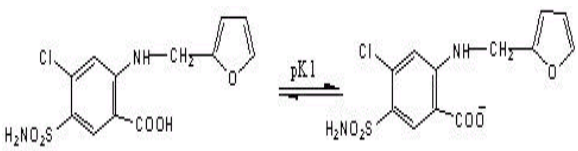
amount of 1M sodium perchlorate solution. The titrations were carried out at 27^{0}C in an inert atmosphere by bubbling oxygen free nitrogen gas through an assembly containing the electrode to expel out CO_2.

The experimental procedure, in the study of ternary chelated by the potentiometric titration technique, involves the titration of carbonate free solution of in 80 %(v/v) ethanol-water, were corrected by method of Vanuitert and Hass[55]. The formation constant of ternary complexes were determined by computational programmed SCOGS[56, 57] to minimize the standard derivation.

Results and Discussion

Binary metal complexes

The proton ligand constant and metal ligand stability constant of drug furosemide and amino acids with copper (II) determined in 80 % (v/v) ethanol-water mixture at 27°C and ionic strength μ= 0.1 M $NaCl0_4$ are given in **Table I** , already published in research journal[58-60].



(Proton dissociation scheme for free ligand Furosemide in 80% ethanol- water medium)

These values are important for the determination of stability constant of mixed ligand complexes therefore mentioned here.

Table I

Ligands	PK_1	PK_2	Chromium	
			$Logk_1$	$LogK_2$
Furosemide	5.6315	--	2.8309	-
Glycine	2.7700	9.7400	6.5100	3.9400
Leucine	3.8100	10.340	7.7078	4.3500
Glutamic Acid	3.1360	5.8987	3.5087	3.0419
Glutamine	3.0100	9.2800	7.2486	6.0816
Valine	3.2100	9.8024	5.6122	3.5901
Methionine	3.1200	9.6000	3.1000	-
Phenylalanine	3.1400	9.3000	6.4405	5.3616

Ternary metal complexes.

The pH metric titration curves of ternary systems shows that the mixed ligand curve coincide with A+D complex curve up to the pH ~ 3.7 and after this pH, it deviates. Theoretical composite curve remains toward left of the mixed ligand complex curve. After pH ~ 4.5, the mixed ligand curve drifts towards X-axis, indicating the formation of hydroxide species. Since the mixed ligand curve coincide with individual metal complex titration curves, the formation of 1:1:1 complex by involving stepwise equilibrium.

The primary ligand drug furosemide form 1:1 and secondary ligand amino acid glycine form 1:1 and 1:2 complexes with Cu(II). It is evident from the figure of percentage concentration species of Cu (II) - furosemide –glycine -system that the percentage distribution curves of free metal decreases sharply with increasing pH. This indicates involvement of metal ion in the complex formation process. Percentage concentration of free ligands furosemide and glycine increases and this increase may be due to the dissociation of ligand present in the system, as a function of pH.

Species distribution studies:

To visualize the nature of the equilibria and to evaluate the calculated stability constant of ternary complexes Cu (II)-furosemide-glycine, species distribution curves have been plotted as a function of pH at temperature 27^{0}C and μ = 0.1 M $NaClO_4$ by using SCOG programme.

It can be seen that, the concentration of Cu(II)-furosemide-glycine increases from pH~2.5, whereas the concentration for the formation of D(Furosemide) and HR(Glycine) show continuous decrease with increasing pH which indicates the formation of Cu(II)-furosemide-glycine. The concentration of this species continuously increases; confirm the formation of ternary complexes.